D6

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/12, C07K 14/715, 16/28, A61K 38/17 — A1

(11) International Publication Number: WO 00/61757

(43) International Publication Date: 19 October 2000 (19.10.00)

(21) International Application Number: PCT/US00/09699

(22) International Filing Date: 12 April 2000 (12.04.00)

(30) Priority Data:
60/128,849 — 12 April 1999 (12.04.99) — US

(71) Applicant: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080–4990 (US).

(72) Inventors: GODDARD, Audrey; 110 Congo Street, San Francisco, CA 94131 (US). PAN, James; 2705 Coronet Boulevard, Belmont, CA 94002 (US). YAN, Minhong; 1910 Garden Drive #114, Burlingame, CA 94010 (US).

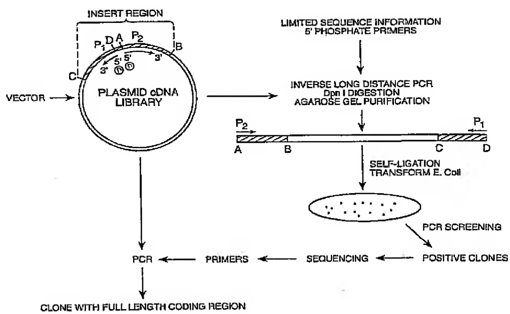
(74) Agents: MARSCHANG, Diane, L. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080–4990 (US).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TUMOR NECROSIS FACTOR HOMOLOGS AND NUCLEIC ACIDS ENCODING THE SAME



(57) Abstract

The present invention is directed to novel polypeptides having homology to members of the tumor necrosis factor receptor family, and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

```
        10          20          30          40          50
MDCQENEYWDQWGRCVTCQRCGPGQELSKDCGYGEGGDAYCTACPPRRYK

        60          70          80          90         100
SSWGHHRCQSCITCAVINRVQKVNCTATSNAVCGDCLPRFYRKTRIGGLQ

       110         120         130         140         150
DQECIPCTKQTPTSEVQCAFQLSLVEADAPTVPPQEATLVALVSSLLVVF

       160         170         180         190         200
TLAFLGLFFLYCKQFFNRHCQRVTGGLLQFEADKTAKEESLFPVPPSKET

       210         220         230         240         250
SAESQVSENIFQTQPLNPILEDDCSSTSGFPTQESFTMASCTSESHSHWV

       260         270         280         290
HSPIECTELDLQKFSSSASYTGAETLGGNTVESTGDRLELNVPFEVPSP
```

FIG._2

```
        10          20          30          40          50
MDCQENEYWDQWGRCVTCQRCGPGQELSKDCGYGEGGDAYCTACPPRRYK

        60          70          80          90         100
SSWGHHRCQSCITCAVINRVQKVNCTATSNAVCGDCLPRFYRKTRIGGLQ

       110         120         130         140         150
DQECIPCTKQTPTSEVQCAFQLSLVEADAPTVPPQEATLVALVSSLLVVF

       160         170         180         190         200
TLAFLGLFFLYCKQFFNRHCQRGGLLQFEADKTAKEESLFPVPPSKETSA

       210         220         230         240         250
ESQVSENIFQTQPLNPILEDDCSSTSGFPTQESFTMASCTSESHSHWVHS

       260         270         280         290
PIECTELDLQKFSSSASYTGAETLGGNTVESTGDRLELNVPFEVPSP
```

FIG._4

the mammalian proteins referred to as FADD/MORT1, TRADD, and RIP [Cleaveland and Ihle, Cell, 81:479-482 (1995)].

Upon ligand binding and receptor clustering, TNFR1 and CD95 are believed to recruit FADD into a death-inducing signaling complex. CD95 purportedly binds FADD directly, while TNFR1 binds FADD indirectly via TRADD [Chinnaiyan et al., Cell, 81:505-512 (1995); Boldin et al., J. Biol. Chem., 270:387-391 (1995); Hsu et al., supra; Chinnaiyan et al., J. Biol. Chem., 271:4961-4965 (1996)]. It has been reported that FADD serves as an adaptor protein which recruits the *Ced-3*-related protease, MACHα/FLICE (caspase 8), into the death signaling complex [Boldin et al., Cell, 85:803-815 (1996); Muzio et al., Cell, 85:817-827 (1996)]. MACHα/FLICE appears to be the trigger that sets off a cascade of apoptotic proteases, including the interleukin-1β converting enzyme (ICE) and CPP32/Yama, which may execute some critical aspects of the cell death program [Fraser and Evan, supra].

It was recently disclosed that programmed cell death involves the activity of members of a family of cysteine proteases related to the *C. elegans* cell death gene, *ced-3*, and to the mammalian IL-1-converting enzyme, ICE. The activity of the ICE and CPP32/Yama proteases can be inhibited by the product of the cowpox virus gene, *crmA* [Ray et al., Cell, 69:597-604 (1992); Tewari et al., Cell, 81:801-809 (1995)]. Recent studies show that CrmA can inhibit TNFR1- and CD95-induced cell death [Enari et al., Nature, 375:78-81 (1995); Tewari et al., J. Biol. Chem., 270:3255-3260 (1995)].

As reviewed recently by Tewari et al., TNFR1, TNFR2 and CD40 modulate the expression of proinflammatory and costimulatory cytokines, cytokine receptors, and cell adhesion molecules through activation of the transcription factor, NF-KB [Tewari et al., Curr. Op. Genet. Develop., 6:39-44 (1996)]. NF-κB is the prototype of a family of dimeric transcription factors whose subunits contain conserved Rel regions [Verma et al., Genes Develop., 9:2723-2735 (1996); Baldwin, Ann. Rev. Immunol., 14:649-681 (1996)]. In its latent form, NF-κB is complexed with members of the IκB inhibitor family; upon inactivation of the IκB in response to certain stimuli, released NF-κB translocates to the nucleus where it binds to specific DNA sequences and activates gene transcription.

For other recent reviews of such signaling pathways see, e.g., Ashkenazi et al., Science, 281:1305-1308 (1998) and Nagata, Cell, 88:355-365 (1997).

SUMMARY OF THE INVENTION

Applicants have identified cDNA clones that encode novel polypeptides having certain sequence identity to previously-described tumor necrosis factor receptor protein(s), wherein the polypeptides are designated in the present application as "DNA98853" polypeptide and "DNA101848" polypeptide.

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a DNA98853 polypeptide. In certain aspects, the isolated nucleic acid comprises DNA encoding the DNA98853 having amino acid residues 1 to 299 or 1 to 136 of Figure 2 (SEQ ID NO:3), or is complementary to such encoding nucleic acid sequences, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. The isolated nucleic acid sequence may comprise the cDNA insert of the vector deposited on April 6, 1999 as ATCC 203906 which includes the nucleotide sequence encoding DNA98853 polypeptide.

In another embodiment, the invention provides a vector comprising DNA encoding a DNA98853 polypeptide. A host cell comprising such a vector is also provided. By way of example, the host cells may be

CHO cells, *E. coli*, or yeast. A process for producing DNA98853 polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of DNA98853 polypeptide and recovering DNA98853 polypeptide from the cell culture.

In another embodiment, the invention provides isolated DNA98853 polypeptide. In particular, the invention provides isolated native sequence DNA98853 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 299 of Figure 2 (SEQ ID NO:3). Additional embodiments of the present invention are directed to isolated extracellular domain sequences of a DNA98853 polypeptide comprising amino acids 1 to 136 of the amino acid sequence shown in Figure 2 (SEQ ID NO:3), or fragments thereof. Optionally, the DNA98853 polypeptide is obtained or is obtainable by expressing the polypeptide encoded by the cDNA insert of the vector deposited on April 6, 1999 as ATCC 203906.

In another embodiment, the invention provides chimeric molecules comprising a DNA98853 polypeptide or extracellular domain sequence or other fragment thereof fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises a DNA98853 polypeptide fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to a DNA98853 polypeptide or extracellular domain thereof. Optionally, the antibody is a monoclonal antibody.

In a still further embodiment, the invention provides diagnostic and therapeutic methods using the DNA98853 polypeptide or DNA encoding the DNA98853 polypeptide.

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a DNA101848 polypeptide. In certain aspects, the isolated nucleic acid comprises DNA encoding the DNA101848 polypeptide having amino acid residues 1 to 297 or 1 to 136 of Figure 4 (SEQ ID NO:6), or is complementary to such encoding nucleic acid sequences, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. The isolated nucleic acid sequence may comprise the cDNA insert of the vector deposited on April 6, 1999 as ATCC 203907 which includes the nucleotide sequence encoding DNA101848 polypeptide.

In another embodiment, the invention provides a vector comprising DNA encoding a DNA101848 polypeptide. A host cell comprising such a vector is also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing DNA101848 polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of DNA101848 polypeptide and recovering DNA101848 polypeptide from the cell culture.

In another embodiment, the invention provides isolated DNA101848 polypeptide. In particular, the invention provides isolated native sequence DNA101848 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 297 of Figure 4 (SEQ ID NO:6). Additional embodiments of the present invention are directed to isolated extracellular domain sequences of a DNA101848 polypeptide comprising amino acids 1 to 136 of the amino acid sequence shown in Figure 4 (SEQ ID NO:6), or fragments thereof. Optionally, the DNA101848 polypeptide is obtained or is obtainable by expressing the polypeptide encoded by the cDNA insert of the vector deposited on April 6, 1999 as ATCC 203907.

In another embodiment, the invention provides chimeric molecules comprising a DNA101848 polypeptide or extracellular domain sequence or other fragment thereof fused to a heterologous polypeptide

or amino acid sequence. An example of such a chimeric molecule comprises a DNA101848 polypeptide fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to a DNA101848 polypeptide or extracellular domain thereof. Optionally, the antibody is a monoclonal antibody.

In a still further embodiment, the invention provides diagnostic and therapeutic methods using the DNA101848 polypeptide or DNA encoding the DNA101848 polypeptide.

Applicants have surprisingly found that the TNF family ligand referred to as EDA-A2 binds to the DNA101848 receptor. The present invention thus provides for novel methods of using antagonists or agonists of these TNF-related ligand and receptors. The antagonists and agonists described herein find utility for, among other things, *in vitro*, *in situ*, or *in vivo* diagnosis or treatment of mammalian cells or pathological conditions associated with the presence (or absence) of EDA-A2.

The methods of use include methods to treat pathological conditions or diseases in mammals associated with or resulting from increased or enhanced EDA-A2 expression and/or activity. In the methods of treatment, EDA-A2 antagonists may be administered to the mammal suffering from such pathological condition or disease. The EDA-A2 antagonists contemplated for use in the invention include DNA101848 or DNA98853 receptor immunoadhesins, as well as antibodies against the DNA101848 or DNA98853 receptor, which preferably block or reduce the respective receptor binding or activation by EDA-A2. The EDA-A2 antagonists contemplated or use further include anti-EDA-A2 antibodies which are capable of blocking or reducing binding of the ligand to the DNA101848 or DNA98853 receptors. Still further antagonist molecules include covalently modified forms, or fusion proteins, comprising DNA101848 or DNA98853. By way of example, such antagonists may include pegylated DNA101848 or DNA98853 or DNA101848 or DNA98853 fused to heterologous sequences such as epitope tags or leucine zippers.

In another embodiment of the invention, there are provided methods for the use of EDA-A2 antagonists to block or neutralize the interaction between EDA-A2 and DNA101848 or DNA98853. For example, the invention provides a method comprising exposing a mammalian cell to one or more EDA-A2 antagonists in an amount effective to decrease, neutralize or block activity of the EDA-A2 ligand. The cell may be in cell culture or in a mammal, *e.g.* a mammal suffering from, for instance, an immune related disease or cancer. Thus, the invention includes a method for treating a mammal suffering from a pathological condition such as an immune related disease or cancer comprising administering an effective amount of one or more EDA-A2 antagonists, as disclosed herein.

The invention also provides compositions which comprise one or more EDA-A2 antagonists. Optionally, the compositions of the invention will include pharmaceutically acceptable carriers or diluents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) (and complementary sequence (SEQ ID NO:2)) of a native sequence DNA98853 polypeptide cDNA (nucleotides 1-903). Also presented is the position of three cysteine-rich repeats encoded by nucleotides 10-126, 133-252 and 259-357 as underlined. The putative transmembrane domain of the protein is encoded by nucleotides 409-474 in the figure.

Figure 2 shows the amino acid sequence (SEQ ID NO:3) derived from nucleotides 1-900 of the nucleotide sequence shown in Figure 1. A potential transmembrane domain exists between and including amino acids 137 to 158 in the figure.